BIOAVAILABILITY OF HEAVY METALS IN SEDIMENTS FROM GRAND LAKE, OKLAHOMA

By

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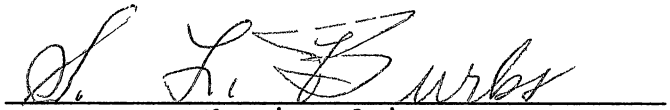

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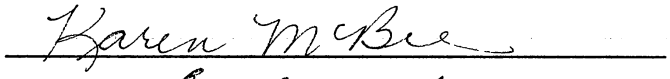

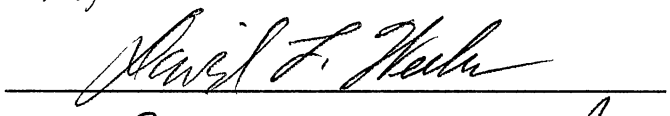

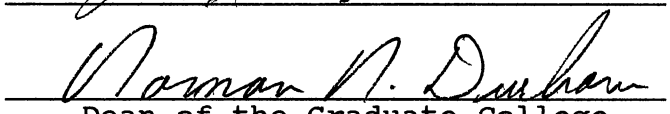

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CHAPTER I

INTRODUCTION

Grand Lake O'the Cherokees is situated in Mayes, Delaware and Ottowa Counties approximately 10 miles east of Vinita and 70 miles northeast of Tulsa, Oklahoma. The lake receives drainage from the Spring and Neosho Rivers. With the close of World War II, mining activity in the Tri-State Mining District made up of Oklahoma, Kansas and Missouri gradually ceased. The abandoned mine shafts filled with water which reacted with iron pyritic minerals to form an acidic solution, with pH values ranging from 3 to 5 [1]. Eventually, the acidic water, laden with heavy metals in solution, flowed out of the mines and reached the surface where it flowed into a tributary of Tar Creek. In 1981, the Tar Creek site was described as one of the nation's most severely polluted sites. The remedial program under Superfund lasted six years and consisted of efforts to plug and cap abandoned water wells. Diversion of flows around sinkholes and mine cave-ins was also part of the clean up.

Aggus, et al. [2] found that although Tar Creek contributed the highest concentration of metals to Neosho River and Grand Lake, Spring River transported the largest

total load into Grand Lake, due to its greater discharge volume. The Galena, Kansas site, also a Superfund site, is the source of dissolved mine tailings which find their way into Grand Lake near Wyandotte, OK, via Spring River. The study also found a decrease in the heavy metal concentration downlake from the confluence of Spring River, implying that a greater percentage of sedimentation occurs at the upper end of the lake. The impact of future deposition in Grand Lake has yet to be assessed.

McCormick [3] in a previous study, analyzed sediment cores from the mouth of Tar Creek, the Tar Creek-Neosho River confluence and the upper end of Grand Lake for heavy metals. Elevated concentrations of some metals were found in all three sites. However, in an acute assay with Daphnia magna of leachate from sediment extracted at pH 6, no toxicity was observed for Grand Lake sediment while sites 1 and 2 produced significant toxicity.

Dawson et al. [4] evaluated the developmental toxicity of sediment collected from two similar sites, Tar Creek and the Neosho River with frog and fathead minnow embryo-larval teratogenesis assays. Levels of metals in the sediment suggested that zinc was the major developmental toxicant. It was found that the EC50 (malformation of 50% of the fish embryos) in the extracts was 0.5 - 1.4 mg/l zinc after normalizaton to 100 mg/l hardness [4]. Due to the high concentrations of zinc in Grand Lake sediments this test should be a useful indicator of potential developmental

effects.

The objectives of this study are to:

1) estimate the levels of cadmium, lead and zinc in gizzard shad by liver and kidney analyses via atomic absorption,
2) relate fish residue concentrations with levels of dissolved metals in the water column at surface and bottom depths to quantify the bioavailability of these metals,
3) evaluate the effects of Grand Lake water column samples upon survival and reproduction of Ceriodaphnia dubia, and
4) evaluate the effects of Grand Lake sediment extracts upon survival of Daphnia magna, Hyallela azteca, Ceriodaphnia dubia and survival and teratogenicity of fathead minnow, Pimephales promelas embryos.

CHAPTER II

REVIEW OF THE LITERATURE

Heavy metal contaminants in aquatic systems undergo two major routes of transport: in solution in the water column and in association with suspended particulates. Heavy metals may be associated with particles in the following ways: adsorbed at particle surfaces, carbonate-bound, occluded in iron and or manganese oxyhydroxides, associated with organic matter (living or detrital), sulfide-bound, or matrix-bound [5]. In addition to the suspended particulate phase, metals in natural water systems may be partitioned in two other phases: aqueous, and bottom sediment, all of which may be available to organisms. Sediments can act as temporary or semi-permanent storage phases during these transport processes. In the latter phase, sediments can act as contaminant sources after the water column pollution has declined and the long-term biological effects of this process are not well characterized.

Discussions of the bioavailability of metals must include a description of the various forms taken by the metal. This requires information about the metal content of a particular water sample to be partitioned into dis-

solved and suspended metal loads.

The total metal concentration in aquatic systems is made up of ionic, colloidal, complexed and particulate forms. Two analytical techniques may be applied to the problem of metal speciation, anodic stripping voltammetry and ultrafiltration and dialysis. The former separates metal species into electroactive, (aqueous ions and labile complexes) and electroinactive (organic complexes and colloidal species) components. Filtration or dialysis separates metal species based on size. Conventionally, the portion passing through a 0.45 um diameter membrane filter is considered to contain the free metal ion and small complexes with organic ligands such as amino, fulvic and humic acids.

It is this latter portion of free ions and weakly complexed species that is considered to be bioavailable while the non-labile portion of inert metal complexes is considered to be biologically unavailable [6]. Thus, the availability of heavy metals for biota is closely related to the chemical species both in solution and in particulate matter. Little is known, however, about the chemical association of metals in suspended materials and sediments.

Recent data concerning the toxicity of metals to aquatic organisms show effect levels over many orders of magnitude of <u>total</u> metal load, suggesting that total metal content is not an indicator of metal bioavailability. Instead, metal toxicity in an aquatic system is usually a

function of the free or ionic metal form and some hydrolyzed species. In sediment, the issue of bioavailability becomes more complex.

In any case, for benthic invertebrates such as C. tentans and H. azteca, toxic effects can be expected to occur only if the chemical concentration is high enough in the sediments such that the equilibrium interstitial water concentration due to desorption is equal to or greater than the concentration demonstrated to cause an effect in a water exposure sediment-free test [7].

Sediments may be characterized with respect to metal speciation. Methods include fractionation by size and physicochemical methods. The metal oxide, organic calcium carbonate coatings or phases of sediment, along with ion exchange sites, are responsible for the sorption of metal ions from solution.

Adding to the difficulty of measuring sediment toxicity, it has been found that contaminants sorbed to naturally aged sediments have a readily desorbable labile fraction and a fraction resistant to equilibrium. This latter fraction requires a longer period of time to reach desorption equilibrium than lab-spiked sediments [8].

Jenne and Luoma, [9] in a study of the particulate phase, reviewed the physicochemical partitioning of metals, in particular, cadmium. It was suggested that the most likely sinks for this metal were oxides and organic substances. They also found that the bioavailability of

cadmium is controlled by the equilibrium concentrations in the sediment-water interface. This equilibrium is maintained by sorption-desorption and dissolution-precipitation reactions.

McCormick [3] obtained sediment leachates from Grand Lake sediment samples extracted with reconstituted water at pH values of 3, 4, 5, 6, 7, 8 and 9. McCormick found that lead extractability was least sensitive to pH while zinc extractability was very sensitive.

Releases of metals from sediment may occur naturally, or as a result of human activity. Examples of the latter include dredging, land disposal of contaminated sediments and pH changes due to acid rain.

Examples of the former cited by Forstner and Prosi [10] include an increase in salinity, of concern in the estuarine environment, a decrease in pH, the introduction of synthetic complexing agents as substitutes for phosphates in detergents, the action of microbes and physical effects such as erosion, dredging and bioturbation. Natural release mechanisms are dependent upon the physicochemical conditions of both the sediment and the water column, since contaminants are released from sedimenting particles during their fall through the water column. Crucial to release processes is the position of the interface between oxic and anoxic strata. In homogeneous aquatic systems, this interface or redoxcline is located in the sediments and in the water column for some stratified lakes [11].

The sediment-water complex can be divided into three layers: the oxic zone, the anoxic zone and the intervening layer, the redoxcline. The oxic zone may extend into the sediment of well-mixed aquatic systems and it is here that degradation of the sediment particles occurs. Oxygen deficiency in sediments leads to dissolution of hydrated manganese oxide, followed by dissolution of iron oxide. In this divalent state, these ions are soluble, as well as any co-precipitates with metallic coatings. Forstner and Prosi [10] found indications that Cu, Zn, and Cd are released from anoxic sediments into surface waters.

Grand Lake exhibits a dimictic type of thermal stratification [12]. During the summer stratification period, the hypolimnion becomes anoxic and the pH is reduced to about 6.0 - 7.0, producing a potential for considerable redissolution of toxic metals from the sediments and later redistribution throughout the lake.

Due to the hardness of the water in Grand Lake and the resulting rapid sedimentation, the system appears to serve as an effective sink for heavy metals. Most of the toxic metals are not very soluble and therefore quickly adsorb onto particulate matter in the impacted ecosystem. As a result of the rapid sedimentation rate in Grand Lake, the water column metal levels rapidly decrease, even close to the source of input. However, intermittent resuspension of the sediments occurs due to flooding of the Neosho and Spring Rivers which can produce currents for several miles

downstream into the lake and result in sediment redistribution [13].

Factors such as this contribute to the problem of determining heavy metal bioavailability in aquatic systems. Since most aquatic organisms are in contact with trace metals in dissolved and particulate forms, accumulation can occur from the water or the solid phases [5]. Thus, the particulate fraction may serve as a significant chronic and acute source of metals to biota. The feeding habits of detritivores and possible physical disturbances such as dredging or seasonal flooding, respectively, account for these potential responses.

In an extensive review on the effects of heavy metal contamination on aquatic organisms, Mance [14] found several trends. First, it was observed that salmonid species are ten times more sensitive to the effects of cadmium than are the non-salmonids. This trend was repeated for the short-term (4-day exposure) effects of zinc, but was contradicted for long-term exposure. Here, non-salmonids were found to be at least as sensitive to the effects of zinc as the salmonids. Mance found little difference in the response of salmonids and non-salmonids to the effects of lead. Also, there appears to be no difference in the toxicity of the various inorganic salts of lead.

Mance [14] found that for all fish species examined, as water hardness (mg/l CaCO3) increases, toxicity decreases. He also found that the adverse effect level

decreases with an increase in the duration of lead and cadmium exposure.

In an assessment of effects on invertebrates, Mance found that crustaceans were most sensitive to lead and cadmium. This class was most commonly represented by D. magna, with little to no information concerning C. dubia. It was found that insect larvae were the least sensitive to the effects of cadmium, with response concentrations corresponding to those of freshwater fish. Studies of the effects of water hardness using Tubifex tubifex and D. magna show that an increase in hardness reduced zinc toxicity, but other studies were inconclusive, or in some cases, even suggested the reverse [14].

Variability among reported effects levels is high for most metals. O'Donnell et al. [15] found a range from 0.01 - 63,500 ug/l in a review of 101 studies of copper toxicity in aquatic systems. Biological, chemical and experimental factors contribute to this variation.

In preparation for an assessment of the acute toxicity of contaminated sediments, Ziegenfuss et al. [16] found D. magna to be more sensitive than Chironomus tentans in seventeen standard acute toxicity tests of organic chemicals and heavy metals without sediment, significantly so for heavy metals. In a sediment toxicity test using both D. magna and C. tentans, the 48-hour LC50's for kepone were calculated for each species based on the chemical concentration in the sediment, the column water and the

sediment interstitial water. The results indicated that the primary exposure was via the water, not the sediments as such. This conclusion was based on the fact that the LC50 values of the water concentrations were about equal with and without sediments [16].

Adams et al. [7] examined the effects of kepone-contaminated sediment on C. tentans. The study concluded that the main route of exposure was from the interstitial water and or the water at the sediment-water interface.

Geisy et al. [17] compared three sediment bioassay techniques using sediments from the Detroit River contaminated with heavy metals and organic compounds. The ability of the D. magna 48-hour lethality assay, the Photobacterium phosphoreum 15-minute bioluminescence inhibition (Microtox) assay and the C. tentans 10-d growth reduction assay to distinguish grades of toxicity was assessed. Of the three, the first two were conducted with sediment pore water and the latter with whole sediment samples.

It was found that the D. magna 48-h acute bioassay was capable of predicting toxicity so great that benthic invertebrates would not be expected to be present. The Microtox assay was found to be the most sensitive and the D. magna assay the least sensitive in distinguishing between grades of sediment toxicity. However, based on lethality, the C. tentans assay was less sensitive than the D. magna assay. Correlations between the results of all the assays existed, but the results of one assay did not

accurately predict the results of the other two.

Bioavailability can best be described using a physiological response of an organism, in this case, sequestering of heavy metals in tissues. Possible tissues to consider include liver, bile duct and gall bladder; previous work found little value in muscle tissue as an indicator [2]. This study also found cadmium, chromium, lead and zinc in the livers of omnivorous and piscivorous fish. At that time no data were available for planktivorous fish [2].

Similar results were found in a study of metal-contaminated lakes in the Sudbury region of northeast Ontario. Analyses of fish tissues revealed that muscle was a poor indicator of increased metal availability. Liver tissue proved to be a good indicator for copper, and kidney tissue for nickel [18].

It has been demonstrated that uptake via the gills is a primary mechanism for the water-soluble fraction of metal contaminants [19, 20]. In heavily polluted aquatic systems with elevated contamination of particles and prey organisms, metal uptake by the intestinal lumen may be of primary importance. Dallinger and Kautzky [21] found evidence that the uptake of heavy metals through a short food chain by rainbow trout, Salmo gairdneri, can be an important factor in the heavy metal budget of the fish.

Theoretically, the main routes of exposure of fish to cadmium would occur through the food, water, or a combination of both. However, Hatakeyama and Yasuno [22] demon-

strated with a combined feeding and exposure to water levels study, that for cadmium, the principal route appears to be via the water. Williams and Giesy [23] found no significant increase in whole-fish cadmium levels in control water regardless of food concentration, whereas fish subjected to 10 ug/l in the water had significantly higher cadmium residues than the control. That the gills are the primary site of uptake is supported by several studies [19, 24]. Accumulation of cadmium within specific tissues once uptake occurs has also been well documented [25-28]. These authors found that cadmium was principally distributed in the kidney, liver and gills.

Excretion of heavy metals in vertebrates occurs mainly through renal and biliary pathways. Factors affecting excretion of heavy metals include chelating agents, synergistic effects, fluctuations in acid-base equilibria, nutritive status, parasite load, or otherwise poor environmental conditions. Since these same factors affect the excretion of essential metals, any change in homeostasis may indicate concentration changes in these metals as well.

A study by Grahl et al. [29] on the excretion of heavy metals by fish, tested the utility of fish bile as an indicator of environmental toxicants and for identification of chronic heavy metal intoxication. These heavy metal complexes usually occur as low-molecular weight compounds while higher molecular weight compounds such as metallothioneines are filtered by glomeruli but then undergo reab-

sorption. Gel-permeation studies find evidence of higher-molecular weight compounds in the bile.

Although analysis for the presence of metallothionein has been suggested by Roch et al. [30] as an alternative indicator of heavy metals, other data show that in the natural environment, two low-molecular weight non-metallothionein proteins are involved in the detoxification of cadmium. A study by Thomas et al. [20] found that at relatively low levels of cadmium such as in natural waters, two proteins in the liver and kidney were active in sequestering the cadmium while metallothioneins in the liver were not activated except at very high levels, ie. 1000 ug/ml.

Because of difficulties described previously there can be no universally accepted scale for monitoring contamination by metal residues in fish. Applications on a local scale and in particular, in long-range studies, seem more appropriate.

Given the preceding observations, analyses of tissues such as liver, kidney, and gill of fish seems to be the most appropriate monitor for the presence of low-level chronic metal contaminants. To estimate the bioavailability of these contaminants in Grand Lake, metal levels in tissues of fish collected from the lower end will be compared with those from the upper end of the lake. Gizzard shad, Dorosoma cepedianum are relatively territorial and thus, spend a majority of their life cycle in a relatively small area of the lake. Shad are filter-feeders, straining

detritus from the bottom and plankton from the water. Analysis of liver and kidney tissue will provide a means of estimating recent exposure.

Since a similar, previous study [2] was done in 1982, further research based on the same parameters should provide some insight into the long-term effects of heavy metals loading on the fish of this aquatic system. Also, background data have been accumulated on the metal concentrations at different depths of Grand Lake since that period.

Most criteria for assessing the aquatic environment have been based on aqueous concentrations in the water column. However, sediment quality may also affect aquatic life and criteria have recently been developed to assess these effects.

One approach involves the concept of the sediment quality triad [31] developed by Chapman which incorporates *in situ* studies, sediment bioassays and sediment chemistry. When applied to the present study, incorporation of *in situ* bioaccumulation levels with results of laboratory bioassays on natural sediments and results of sediment chemical analysis should provide an estimate of whether or not the metals in Grand Lake sediments are detrimentally bioavailable.

CHAPTER III

SAMPLING LOCATIONS

Water and sediment samples

Water and sediment samples were collected from four previously established sampling stations selected by the Grand River Dam Authority.

GRDA #1 (Station 1) was located approximately 40 miles upstream from the Pensacola Dam and approximately 2.5 miles downstream of the confluence of the Spring and Neosho Rivers. Maximum depth was 45 feet and the shoreline was steep with abundant vegetation.

GRDA #2 (Station 2) was located underneath Sailboat Bridge, approximately 23.5 miles upstream of the Pensacola Dam. Maximum depth was 70 feet and the shoreline was relatively flat with plentiful vegetation.

GRDA #3 (Station 3) was located near Two Tree Island, approximately 11.5 miles upstream from the Pensacola Dam. Maximum depth was 112 feet. The shoreline was extensively developed with residential areas just above the flood plain.

GRDA #4 (Station 4) was located approximately 1 mile upstream of the Pensacola Dam with a maximum depth of 112 feet.

Fish collection sites

Fish were collected from Stations 1 and 4 to compare heavy metal residue levels at the outermost areas in the lake.

CHAPTER IV

MATERIALS AND METHODS

METALS ANALYSIS

Sample handling

All glass and plastic ware used in collection and analysis of water, sediment and fish tissue samples was washed with detergent and rinsed with acid and double-distilled water. Fish samples were dissected as soon as possible after capture and were frozen when circumstances did not permit immediate dissection. Sediment samples were stored at 4 degrees Celcius.

Quality control

In the spectrophotometric analysis for heavy metals of water, sediment and fish tissues, a duplication rate of at least 20% was maintained. Standard practice included analysis of field blanks (for water sample analysis), procedural blanks and EPA quality control reference solutions, including analysis of freeze-dried fish reference tissues.

Water and sediment collection and analysis

Variables measured in the field included turbidity, Secchi disk transparency, conductivity, pH, temperature and dissolved oxygen. Measurements were made with a Hydro-lab Digital 4041, Yellow Springs Instrument dissolved oxygen field meter and turbidity was measured with a HACH turbidimeter. Water samples were collected with an acrylic Van Dorn water sampler for measurement of the following metals: arsenic, cadmium, copper, mercury, lead, iron, zinc and selenium. Samples were filtered through a 0.45 um membrane for analysis of dissolved and suspended metal content. The analyses were performed with a Perkin Elmer Model 5000 Atomic Absorption Spectrophotometer equipped for both flame and graphite furnace analysis. Water samples were collected once a month for four months and sediment samples were collected twice during the same period. Methods for metals analysis were taken from USEPA Methods for the Chemical Analysis of Water and Wastes [32].

Fish collection and analysis

Gizzard shad were collected by personnel of the Oklahoma State University Cooperative Fish and Wildlife Unit via electroshock and gill netting from Station 4 from mid-April to mid-May. Fish from Stations 1 and 2 were collected by throw net in mid-September by a local fisherman.

All analyses of liver and kidney tissue were performed via atomic absorption spectrophotometry after acid diges-

tion. Individual organs were weighed to 5 decimal places on a Mettler H20T analytical balance. Tissues and sediment were digested according to USEPA's Method 3050 [33] and can be summarized as follows: A homogeneous 0.1 - 2.0g sample (wet weight) was digested with concentrated nitric acid and hydrogen peroxide. The digestate was refluxed with nitric acid and diluted to the appropriate volume with 0.2 N nitric acid (depending on the original tissue weight).

Necessary reagents included double distilled water, reagent grade concentrated nitric acid and 30% hydrogen peroxide.

SEDIMENT EXTRACT BIOASSAYS

Sample collection

Sediment samples were collected with an Ekman dredge at the four main stations described previously, GRDA #'s 1 - 4. Several grabs were made along a transect at each location and a composite prepared on site in polyethylene buckets. The composite sediment samples were stored in polyethylene bottles and iced immediately. Aliquots were taken for metals analysis and extract preparation.

Laboratory Control

For each assay, a laboratory control of Hard Reconstituted Water (recon) was tested concurrently. Recon was prepared by adding measured amounts of NaHCO3, $CaSO_4 \cdot H_2O$, $MgSO_4$, and KCl to deionized distilled water in accordance

with USEPA procedures [34]. Hard Recon has a pH of about 7.6 - 8.0, an alkalinity of 110 - 120 and a hardness of about 160 - 180, both measured as mg/l of $CaCO_3$.

Extract preparation

Sediment extracts were prepared to investigate potential effects upon two species of daphnids, one species of amphipod and fathead minnow embryos. A measured portion of the sediment was treated at pH 4, 8 and 10 and tumbled for 24 hours in either Grand Lake column water from the appropriate station or reconstituted water of the appropriate hardness. The extracts were contained in polyethylene bottles and tumbled in a Rotatox tumbling unit. A 1:4 sediment to water ratio was maintained for all extract preparation. At 1, 4, 12 and 23 hours, the pH was monitored and readjusted if necessary. At the end of the 24-hour period, the pH for all samples was adjusted to pH 8 and either centrifuged for 15 minutes at 10,000 rpm or allowed to settle overnight before introduction of the test organisms.

7-d Ceriodaphnia dubia Survival and Reproduction Test

This assay was performed according to USEPA's Method 1002.0 [34]. Less than 24-hour old neonates were used. Endpoints compared were survival and reproduction. Test water was renewed daily and neonates counted and removed. Mean total numbers of young produced at the end of the 7-d

3-brood period were compared. See Table 1 for a summary of test conditions. Grand Lake column water samples collected approximately half a meter below the surface of Stations 1 - 4 were tested.

Table 1. Conditions for 7-d C. dubia Survival and Reproduction Assay.

1.	Test type:	static renewal
2.	Temperature:	26.0 ± 1.0 °C
3.	Light quality:	ambient laboratory illumination
4.	Light intensity:	10 - 20 uE/m^2/s
5.	Photoperiod:	16 h light, 8 h dark
6.	Test chamber size:	30 ml
7.	Test solution volume:	15 ml
8.	Renewal of test solutions:	daily
9.	Age of test organisms:	<24 h, and released within an 8-h period
10.	No. neonates per chamber:	1
11.	No. replicate test chambers:	10
12.	Feeding regime:	fed 0.1 ml each of TCY and algal suspension daily
13.	Aeration:	none
14.	Control Water:	Hard Reconstituted Water
15.	Samples tested:	Grand Lake column water from four stations collected approximately half a meter below the surface

96-h D. magna survival assay

After the 24-hour tumbling period, sediment extracts were adjusted to pH 8 and a 500-ml aliquot of each extract poured into 4 250-ml polycarbonate centrifuge bottles and centrifuged for 15 minutes at 10,000 rpm. Three of the bottles containing 100 ml each were used as replicates in a 96-h D. magna toxicity test. Eight juvenile D. magna were used per replicate. The organisms were fed one drop of TCY digest per bottle on Days 0 and 2 of the test. At the end of the 96-h period, the overlying water was filtered through a fine mesh screen and the organisms recovered and counted.

The overlying water, about 200 ml, in the remaining centrifuge tube was used in a teratogenicity assay, monitored for physical-chemical parameters and a 100-ml aliquot filtered for suspended and dissolved metal levels. At the end of the 96-h test period, overlying water from the three replicates was combined for measurment of physical-chemical parameters.

H. azteca and C. dubia

48-hour assays

In these assays, only sediment extracts from Stations 1 and 4 were tested. Grand Lake column water was used in a 1:4 sediment to water ratio. The mixture was tumbled as before and all extracts adjusted to pH 8 at the end of the 24-hour tumbling period.

Fifteen ml of the extract were poured into 30-ml plas-

tic containers for the C. dubia assay and 10 ml per plastic petri dish for the H. azteca assay. The extracts were allowed to settle overnight before introduction of the test organisms. Less than 24-h old C. dubia neonates and 1-2 week old H. azteca juveniles were used.

Lack of clarity in the extracts tumbled at pH 8 and 10 prevented an accurate count on Day 1 of the test. Upon termination of the test, the extract was poured through a fine mesh screen to recover the organisms.

Fathead Minnow 7-d Embryo-Larval Survival and Teratogenicity Assay.

This assay was performed according to USEPA's Method 1001.0 [34]. Fathead minnow embryos were exposed to sediment extracts from four lake stations for seven days in a static renewable test. On days 2, 4 and 6, the water was renewed. Once a day, the test chambers were cleaned by removal of dead organisms and egg cases from recently hatched larvae. Only those organisms with gross physical deformities such as lack of appendages, lack of fusiform shape, lack of mobility or other survival-limiting characteristics were considered abnormal and counted as dead. Endpoints compared in this test included total percent mortality, combined number of dead embryos and dead and deformed larvae. See Table 2 for conditions employed in this assay.

Table 2. Conditions for the Fathead Minnow (P. promelas) Embryo-Larval Survival and Teratogenicity Test

1.	Test type:	static renewal
2.	Temperature:	26.0 ± 1.0 °C
3.	Light quality:	ambient laboratory illumination
4.	Light intensity:	10 - 20 uE/m^2/s
5.	Photoperiod:	16 h light, 8 h dark
6.	Test chamber size:	25 ml
7.	Test solution volume:	8 ml
8.	Renewal of test solutions:	every other day
9.	Age of test organisms:	<36 h
10.	No. embryos per chamber:	8
11.	No. replicate test chambers:	3
12.	No embryos per sample	24
13.	Feeding regime:	none required
14.	Aeration:	aerated for 30 minutes before intiation of test
15.	Control Water:	Hard Reconstituted Water
16.	Samples Tested:	sediment from 4 stations extracted at pH 4, 8 and 10

Statistical analyses

All statistical analyses were performed with the aid of TOXSTAT, a statistical software package [35]. Shapiro-Wilks Test (p=0.01) and Bartlett's Test were used to test for normality and homogeneity of variance, respectively. All percent survival or percent mortality data were transformed (arc-sine) before analysis. Reproduction data for the 7-day C. dubia assay were compared with a non-parametric method, Steel's Many-One Rank Test (a=0.05). All other

comparisons were made with Tukey's Test or Mean Comparison ($p=0.05$).

CHAPTER V

RESULTS AND DISCUSSION

BIOASSAYS

7-d C. dubia Survival and Reproduction Assay

Ten replicates per sample of column water were used. The average number of young produced at the end of 7 days was 21.5 for the control and ranged from 19.5 to 24.6 for the four samples tested. No significant difference in survival or reproduction was detected when the control was compared against lake samples (Table 3).

Table 3. C. dubia survival and reproduction data

Sample Station	Total Tested	No. Surviving	Mean No. of young	SD
Hard Recon	10	10	21.5	1.96
1 Surface	10	10	19.5	2.64
2 Surface	10	9	23.3	3.74
3 Surface	10	9	24.6	4.81
4 Surface	10	10	20.1	7.70

48-h C. dubia and H. azteca assays

Ten replicates per sample for C. dubia and 3 replicates per sample for H. azteca were employed in these assays. Samples tested included a control of untreated hard recon and hard recon and sediment from Stations 1 and 4 extracted at pH 4, 8 and 10. Since the extracts were prepared with Grand Lake column water, blanks consisting of column water from Stations 1 and 4 were also tested. Fisher's Exact Test [35] showed no significant difference when compared to the control.

96-h D. magna survival assay

Percent survival data for three replicates of eight organisms each were averaged and compared using Tukey's Method of Multiple Comparisons after arc-sine transformation [35]. When extracts from sediment from Stations 2 and 3 were compared, no significant difference was found. When extracts from Stations 1 and 4 were compared, sediment from Station 4 extracted at pH 4 produced a mean of 83 percent mortality and was significantly different from the control and all other groups. Survival for the laboratory control was 96 percent and ranged from 91.7 - 75.3 percent for the recon blanks (Table 4).

Table 4. Summary of results of 96-h D. magna sediment extract survival assay

Sample (pH)	Fraction Survival: Mean Transformed	Mean Original	Significance
[a]Hard Recon (I)	1.334	0.960	
Hard Recon (4)	1.160	0.837	
Hard Recon (8)	1.278	0.917	
Hard Recon (10)	1.060	0.753	
Station 1 (4)	1.278	0.917	
Station 1 (8)	1.393	1.000	
Station 1 (10)	1.278	0.917	
Station 2 (4)	1.334	0.960	
Station 2 (8)	1.393	1.000	
Station 2 (10)	1.393	1.000	
Station 3 (4)	1.393	1.000	
Station 3 (8)	1.278	0.917	
Station 3 (10)	1.334	0.960	
Station 4 (4)	0.420	0.170	*
Station 4 (8)	1.393	1.000	
Station 4 (10)	1.393	1.000	

[a]Laboratory control
*Significant at p = 0.05

7-d Fathead Minnow Survival

and Teratogenicity Assay

Three replicates of eight embryos each were used per sample. Tukey's Method yielded no significant differences between groups when recon and sediment from Stations 1 and 4 were compared [35]. When the control and sediment from Stations 2 and 3 were compared, mean transformed percent mortality for Station 3 sediment treated at pH 10 was significantly greater than percent mortality in the control. However, this observed mortality was probably due to fungal growth in the three replicate test chambers. Fungal growth did not occur in any other extracts or control groups. When compared solely on the basis of pH, mean percent mortality for Station 4 sediment at pH 8 was significantly greater than percent mortality in the control (Table 5). High levels of dissolved cadmium and lead in both groups may be responsible for some toxicity (Tables 10 and 11).

Table 5. Summary of results of 7-d Fathead Minnow survival and teratogenicity sediment extract assay

	Fraction Mortality		
Sample (pH)	Mean Transformed	Mean Original	Significance
[a]H. Recon (I)	0.178	0.000	
H. Recon (4)	0.420	0.170	
Station 1 (4)	0.357	0.127	
Station 2 (4)	0.472	0.210	
Station 3 (4)	0.241	0.043	
Station 4 (4)	0.408	0.337	
[a]H. Recon (I)	0.178	0.000	
H. Recon (8)	0.178	0.000	
Station 1 (8)	0.357	0.127	
Station 2 (8)	0.420	0.170	
Station 3 (8)	0.455	0.210	
Station 4 (8)	0.587	0.310	*
[a]H. Recon (I)	0.178	0.000	
H. Recon (10)	0.241	0.043	
Station 1 (10)	0.241	0.043	
Station 2 (10)	0.559	0.293	
[b]Station 3 (10)	0.637	0.363	
Station 4 (10)	0.603	0.337	

[a]Laboratory control
*Significant at p=0.05
[b]Fungal infection

Table 6. Summary of results of assays

Organism Tested	Length of Exposure	Samples Tested	Endpoints	[a]Significant Toxicity Station (pH)
C. dubia	7-days	Grand L. column, 1 - 4	Survival, Reproduction	none
C. dubia, H. azteca	48-hours	Sediment Extract, 1 and 4	Survival	none
D. magna	96-hours	Sediment Extract, 1 - 4	Survival	4(4)
P. promelas	7-days	Sediment Extract, 1 - 4	Survival, Teratogenicity	[b]3(10) 4(8)

[a]Significant at p=0.05
[b]fungal growth

METAL LEVELS

Values from the USEPA Quality Criteria for Water, 1986 were used in the comparisons of sediment extract and column water levels [36]. Values for the protection of freshwater organisms are applicable to waters with 100 mg/l hardness measured as $CaCO_3$.

Table 7. Summary of USEPA Water Quality Criteria

Element	Ambient Water Quality	[a]Protection of Freshwater Organisms
As	"0"	190 ug/l
Cd	10 ug/l	1.1 ug/l
Cu	1 mg/l	12 ug/l
Fe	0.3 mg/l	1.0 mg/l
Pb	50 ug/l	3.2 ug/l
Se	10 ug/l	35 ug/l
Zn	5 mg/l	320 ug/l

[a]at 100 mg/l hardness
Quality Criteria for Water 1986. USEPA 440/5-86-001

Sediment extracts

Results of metals analyses of sediment extracts show some levels greater than the criterion set forth by the USEPA for the protection of aquatic life. Levels of suspended lead in the set of extracts used in the 48-h C. dubia and H. azteca assays exceed the criterion of 3.2

ug/l. Other metals in excess of the USEPA limits [36] include dissolved cadmium and zinc and suspended zinc, iron and copper (Tables 8 and 9).

Table 8. Levels of zinc, cadmium and lead in Grand Lake sediment extracts used in C. dubia and H. azteca assays

Element	Zn		Cd		Pb	
Units	mg/l		ug/l		ug/l	
[a]Station/ Sample (pH)	Susp.	Diss.	Susp.	Diss.	Susp.	Diss.
Recon (unt.)	0.036	0.013	<0.10	<0.10	<1.50	<1.50
Recon (4)	0.021	0.052	<0.10	0.20	[b]4.86	1.76
Recon (8)	0.013	0.023	<0.10	<0.10	1.87	<1.50
Recon (10)	0.014	0.013	<0.10	0.12	<1.50	<1.50
1 W (4)	0.024	0.023	0.21	0.11	[b]6.23	<1.50
1 W (8)	0.029	0.015	<0.10	<0.10	<1.50	<1.50
1 W (10)	0.063	0.013	<0.10	<0.10	<1.50	<1.50
4 W (4)	0.142	0.066	<0.10	0.15	[b]6.03	3.05
4 W (8)	0.075	0.041	<0.10	0.29	1.61	<1.50
4 W (10)	0.025	0.052	0.11	0.39	<1.50	<1.50
1 S (4)	0.104	[b]0.409	0.17	0.35	[b]3.87	<1.50
1 S (8)	0.254	0.010	0.37	1.10	[b]7.75	<1.50
4 S (4)	0.239	0.142	0.25	[b]1.17	[b]8.38	<1.50
4 S (8)	[b]0.659	0.018	0.66	<0.10	[b]36.07	<1.50

[a]W = column water
S = sediment
[b]Exceed USEPA criteria (Table 7)

Table 9. Levels of iron and copper in Grand Lake sediment extracts used in C. dubia and H. azteca assays

Element	Fe		Cu	
Units	mg/l		ug/l	
[a]Station/ Sample (pH)	Susp.	Diss.	Susp.	Diss.
Recon (unt.)	<0.06	<0.06	1.88	3.56
Recon (4)	<0.06	<0.06	2.20	3.06
Recon (8)	<0.06	<0.06	2.78	3.34
Recon (10)	<0.06	<0.06	1.52	2.29
1 W (4)	0.16	<0.06	2.32	4.56
1 W (8)	<0.06	<0.06	1.93	4.52
1 W (10)	0.14	<0.06	2.32	3.74
4 W (4)	<0.06	<0.06	2.50	4.71
4 W (8)	0.10	<0.06	3.01	4.91
4 W (10)	<0.06	<0.06	2.95	4.93
1 S (4)	0.78	<0.06	5.19	1.14
1 S (8)	[b]6.58	<0.06	6.79	1.74
4 S (4)	[b]4.29	<0.06	6.92	6.55
4 S (8)	[b]27.82	0.27	b22.53	6.75

[a]W = column water
S = sediment
[b]Exceed USEPA criteria (Table 7)

Levels of dissolved metals which exceed USEPA criteria appear to occur more frequently in sediment extracted at pH values of 8 and 10, regardless of location of station on the lake (Tables 10 and 11).

Table 10. Levels of zinc, cadmium and lead in Grand Lake sediment extracts used in D. magna and P. promelas assays

Element	Zn		Cd		Pb	
Units	mg/l		ug/l		ug/l	
Station/ Sample (pH)	Susp.	Diss.	Susp.	Diss.	Susp.	Diss.
Recon (unt.)	0.101	0.033	0.41	0.11	1.86	<1.50
Recon (4)	0.042	0.090	0.14	0.14	2.18	2.15
Recon (8)	0.022	0.010	0.20	0.10	[d]4.02	d4.57
Recon (10)	0.011	0.036	0.18	0.14	2.48	1.61
1 S (4)	0.113	[d]0.476	0.58	0.98	[d]117.96	<1.50
1 S (8)	[d]0.396	0.095	[d]2.26	0.21	[d]30.86	1.60
1 S (10)	0.216	0.105	[d]1.44	[d]1.54	[d]19.95	2.68
2 S (4)	0.074	0.306	0.35	0.54	d5.52	<1.50
2 S (8)	0.307	0.051	0.91	0.24	[d]22.23	<1.50
2 S (10)	[d]0.925	[d]0.398	[d]1.21	0.42	[d]59.74	[d]5.96
3 S (4)	0.062	0.112	0.64	0.14	[d]4.34	<1.50
3 S (8)	0.318	0.093	0.69	0.60	[d]19.03	<1.50
[a]3 S (10)	[d]0.544	[d]1.493	[d]1.77	[d]1.74	[d]29.73	[d]36.23
[b]4 S (4)	0.062	0.163	0.36	0.21	[d]3.91	<1.50
[c]4 S (8)	0.274	0.253	0.71	1.09	[d]23.75	[d]9.55
4 S (10)	[d]0.672	0.199	1.01	[d]1.17	[d]31.67	3.04

[a,c]Significant mortality to fathead minnow embryos
[b]Significant mortality to D. magna
[d]Exceed USEPA criteria (Table 7)

Table 11. Levels of iron and copper in Grand Lake sediment extracts used in D. magna and P. promelas assays

Element	Fe		Cu	
Units	mg/l		ug/l	
Station/ Sample (pH)	Susp.	Diss.	Susp.	Diss.
Recon (unt.)	<0.06	<0.06	6.94	8.13
Recon (4)	<0.06	<0.06	[d]21.59	3.18
Recon (8)	<0.06	<0.06	5.78	2.18
Recon (10)	<0.06	<0.06	4.10	2.82
1 S (4)	[d]3.34	<0.06	6.30	2.89
1 S (8)	[d]21.89	0.16	[d]16.01	8.03
1 S (10)	[d]19.95	0.21	8.50	[d]36.86
2 S (4)	[d]5.48	<0.06	4.12	3.26
2 S (8)	[d]20.33	0.21	[d]16.31	8.95
2 S (10)	[d]100.9	[d]6.50	[d]23.94	[d]42.27
3 S (4)	[d]3.24	<0.06	2.36	4.83
3 S (8)	[d]37.9	0.60	8.26	[d]13.29
[a]3 S (10)	[d]59.7	[d]53.3	[d]22.96	[d]102
[b]4 S (4)	[d]2.18	<0.06	4.82	2.43
[c]4 S (8)	[d]33.3	[d]7.81	10.32	[d]43.03
4 S (10)	[d]71.0	0.64	[d]36.53	[d]20.95

[a,c]Significant mortality to fathead minnow embryos
[b]Significant mortality to D. magna
[d]Exceed USEPA criteria (Table 7)

Hardness measured as mg/l $CaCO_3$ increased in the sediment extracts treated at pH 4, possibly mediating toxicity due to high levels of dissolved metals (Tables 12 and 13).

Table 12. Physical-chemical data for sediment extracts used in C. dubia and H. azteca assays

Sample (pH)	Alkalinity mg/l as Ca CO3	Hardness	Conductivity uohms/cm3	pH S.U.	Diss. Oxygen mg/l	Temp. °C
R. (unt.)	114	142	490	8.2	8.2	26.2
R. (4)	42	154	650	7.8	7.9	26.2
R. (8)	118	150	500	8.2	7.8	26.2
R. (10)	114	108	605	8.2	7.8	26.2
1 W (4)	14	114	495	7.3	7.8	26.2
1 W (8)	80	110	390	8.0	7.9	26.2
1 W (10)	76	106	340	8.0	7.8	26.2
4 W (4)	20	116	405	7.5	8.0	26.2
4 W (8)	72	118	380	8.0	7.9	26.2
4 W (10)	62	60	350	8.0	7.9	26.2
1 S (4)	116	620	2500	7.1	7.4	26.2
1 S (8)	80	160	800	8.1	7.2	26.2
1 S (10)	166	160	560	7.8	5.0	26.2
4 S (4)	84	840	2200	7.5	7.2	26.2
4 S (8)	154	200	500	7.4	4.2	26.2
4 S (10	336	200	800	7.8	1.0	26.2

Table 13. Physical-chemical data for sediment extracts used in *D. magna* and *P. promelas* assays

Sample (pH)	Alkalinity mg/l as Ca CO3	Hardness	Conductivity uohms/cm3	pH S.U.	Diss. Oxygen mg/l	Temp. °C
R. (unt.)	96	140	499	8.4	8.0	24.9
R. (4)	34	130	800	7.8	8.2	24.9
R. (8)	96	134	600	8.4	8.0	24.9
R. (10)	98	100	620	8.5	8.2	24.9
1 S (4)	16	650	2200	7.5	8.0	24.9
1 S (8)	--	108	620	8.1	8.0	24.9
1 S (10)	192	110	1510	8.4	7.8	24.9
2 S (4)	74	1300	4150	7.8	7.9	24.9
2 S (8)	136	90	1000	8.2	7.6	24.9
2 S (10)	148	120	1350	8.1	9.0	24.9
3 S (4)	152	1250	3600	8.1	8.0	24.9
3 S (8)	156	80	600	8.1	8.5	24.9
3 S (10)	124	110	2000	7.8	11.7	24.9
4 S (4)	158	1340	3500	7.5	5.5	24.9
4 S (8)	152	100	600	8.5	6.5	24.9
4 S (10)	148	80	1450	8.3	8.9	24.9

Significantly greater quantities of dissolved metals were leachable from sediments extracted at the higher pH values of 8 and 10, even though the total quantities of metals in the lower portion of the lake are less than in the upper end. This may be due more to the chemical form or species than the actual amounts present. DiToro [37] has recently hypothesized that the quantity of iron sulfide in sediments may be controlling availability of trace metals. Since most toxic metals form insoluble metallic sulfide salts in the presence of ferrous sulfide, high levels of sulfides would prohibit solubilization of toxic metals from sediments into the overlying water column until all the sulfides had either reacted with more electronegative elements or oxidized to sulfates. Since anoxic conditions were observed for bottom water and sediments, most metals would probably remain bound (Appendix - Field Data).

The sediments in the upper end of Grand Lake appear to be strongly reduced, ie., dark brown to black in color with a strong sulfide odor. This condition may result in a stronger sequestering of the toxic metals as insoluble sulfide salts and thus reduce transport throughout the lower portion of Grand Lake. Obviously, some metals are transported to the lower portion of the lake as evidenced by D. magna bioassay results, however, the physical-chemical conditions in the upper end of lake are acting as a sediment trap to greatly reduce the total quantity transported.

Column water

Levels of suspended metals in excess of USEPA criteria occur most frequently for Station 1, below the confluence of the Spring and Neosho Rivers and gradually decrease at the lower stations. Levels of dissolved metals are lower overall than suspended, and again, gradually decrease toward the lower portion of the lake.

Table 14. Levels of suspended metals in Grand Lake column water - Station 1

Date	6-89		7-89		8-89		10-89	
[a]Depth Element	S	B	[b]S	[b]B	S	B	S	B
Fe, mg/l	[c]1.45	[c]2.89	[c]3.58	[c]4.42	[c]1.03	[c]3.11	0.55	0.70
Cd, ug/l	0.19	0.45	<0.10	<0.10	<0.10	0.12	0.21	0.23
Pb, ug/l	<1.00	3.06	[c]7.51	[c]4.54	[c]2.74	[c]5.98	<1.00	1.9
Zn, mg/l	0.03	0.07	0.15	0.12	0.05	0.14	0.04	[c]0.71
Cu, ug/l	2.96	4.72	5.24	3.38	5.43	9.49	2.10	2.49
As, ug/l	<1.5	3.12	5.42	4.74	4.78	4.54	<1.5	<1.5
Se, ug/l	7.32	4.58	5.68	5.61	4.62	7.02	9.08	11.18

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]mean of triplicate samples
[c]Exceed USEPA criteria (Table 7)

Table 15. Levels of suspended metals in Grand Lake column water - Station 2

Date	6-89		7-89		8-89		10-89	
[a]Depth Element	[b]S	[b]B	S	B	S	B	S	B
Fe, mg/l	0.62	[c]1.33	0.46	[c]2.85	0.23	[c]3.19	0.41	[c]4.34
Cd, ug/l	0.60	0.27	0.41	0.30	<0.10	<0.10	0.22	0.54
Pb, ug/l	<1.0	2.95	<1.0	[c]3.20	<1.0	2.11	2.01	[c]7.68
Zn, mg/l	0.03	0.10	0.16	0.08	0.05	0.09	0.05	0.12
Cu, ug/l	5.47	8.98	2.97	4.82	3.11	4.13	2.40	6.24
As, ug/l	3.27	3.51	4.48	4.24	4.56	3.96	<1.5	<1.5
Se, ug/l	4.18	5.06	5.00	4.94	5.68	5.14	4.18	13.96

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]mean of triplicate samples
[c]Exceed USEPA criteria (Table 7)

Table 16. Levels of suspended metals in Grand Lake column water - Station 3

Date	6-89		7-89		8-89		10-89	
[a]Depth Element	S	B	S	B	S	B	S	B
Fe, mg/l	0.17	0.46	0.27	0.83	0.07	0.28	0.25	0.24
Cd, ug/l	[b]1.83	0.64	<0.10	<0.10	<0.10	<0.10	0.63	0.28
Pb, ug/l	<1.0	[b]3.28	<1.0	<1.0	1.81	<1.0	[b]5.13	[b]8.17
Zn, mg/l	0.05	0.30	<0.01	0.07	0.02	0.01	0.07	0.21
Cu, ug/l	7.59	7.41	1.85	2.36	2.58	3.63	4.24	1.08
As, ug/l	3.28	3.46	4.30	4.22	4.06	<1.5	<1.5	--
Se, ug/l	6.68	7.56	6.16	7.24	6.10	5.66	5.56	--

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]Exceed USEPA criteria

Table 17. Levels of suspended metals in Grand Lake column water - Station 4

Date	6-89		7-89		8-89		10-89	
[a]Depth Element	S	B	S	B	[b]S	[b]B	[b]S	[b]B
Fe, mg/l	<0.06	0.35	0.35	0.17	0.09	0.27	0.16	[c]1.11
Cd, ug/l	0.49	0.47	0.10	<0.10	<0.10	<0.10	0.42	0.28
Pb, ug/l	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	2.10
Zn, mg/l	0.07	0.03	0.03	0.09	0.03	0.03	0.04	0.08
Cu, ug/l	4.07	4.77	4.70	2.01	3.79	2.55	2.68	4.28
As, ug/l	3.64	3.70	4.26	4.12	<1.5	<1.5	<1.5	<1.5
Se, ug/l	7.74	7.18	5.32	5.36	<2.0	<2.0	6.90	6.74

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]mean of triplicate samples
[c]Exceed USEPA criteria (Table 7)

Table 18. Levels of dissolved metals in Grand Lake column water - Station 1

Date	6-89		7-89		8-89		10-89	
[a]Depth Element	S	B	[b]S	[b]B	S	B	S	B
Fe, mg/l	0.06	0.09	0.14	0.24	0.11	0.11	0.16	0.12
Cd, ug/l	[c]1.56	0.72	0.02	<0.10	<0.18	<0.10	<0.10	<0.10
Pb, ug/l	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	[c]26.03	[c]15.04
Zn, mg/l	0.02	0.06	0.02	0.03	0.05	0.07	0.09	0.10
Cu, ug/l	2.97	[c]13.09	5.85	5.0	3.27	3.14	[c]47	[c]40
As, ug/l	3.12	3.48	4.09	4.08	3.92	5.44	<1.5	<1.5
Se, ug/l	8.92	8.92	8.95	9.18	16.70	22.76	15.76	13.46

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]mean of triplicate samples
[c]Exceed USEPA criteria (Table 7)

Table 19. Levels of dissolved metals in Grand Lake column water - Station 2

Date	6-89		7-89		8-89		10-89	
[a]Depth Element	[b]S	[b]B	S	B	S	B	S	B
Fe, mg/l	0.07	0.13	0.02	<0.06	<0.06	<0.06	0.59	0.21
Cd, ug/l	0.20	0.60	<0.10	<0.10	<0.10	<0.10	0.18	0.14
Pb, ug/l	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	[c]12.43	1.88
Zn, mg/l	0.03	0.05	0.01	0.11	0.03	0.02	0.09	0.04
Cu, ug/l	3.56	4.88	3.94	1.03	2.64	3.85	24.47	5.22
As, ug/l	3.38	3.01	4.46	4.48	3.60	5.30	<1.5	<1.5
Se, ug/l	8.93	7.39	7.70	9.30	11.48	12.62	12.86	18.78

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]mean of triplicate samples
[c]Exceed USEPA criteria (Table 7)

Table 20. Levels of dissolved metals in Grand Lake column water - Station 3

Date	6-89		7-89		8-89		10-89	
Depth Element	S	B	S	B	S	B	S	B
Fe, mg/l	0.32	<0.06	<0.06	0.87	<0.06	<0.06	0.10	<0.06
Cd, ug/l	0.47	0.25	0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Pb, ug/l	<1.0	<1.0	<1.0	[b]3.62	<1.0	<1.0	1.48	<1.0
Zn, mg/l	0.05	0.03	0.01	0.08	0.02	0.05	0.04	0.01
Cu, ug/l	3.21	2.55	2.28	9.67	3.22	2.28	2.68	1.98
As, ug/l	4.48	3.92	4.54	4.64	<1.5	<1.5	<1.5	--
Se, ug/l	9.76	9.86	8.32	9.68	13.62	12.48	14.38	--

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]Exceed USEPA criteria (Table 7)

Table 21. Levels of dissolved metals in Grand Lake column water - Station 4

Date	6-89		7-89		8-89		10-89	
[a]Depth Element	S	B	S	B	[b]S	[b]B	[b]S	[b]B
Fe, mg/l	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06	[c]1.03	0.24
Cd, ug/l	<0.10	0.30	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Pb, ug/l	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Zn, mg/l	0.03	0.03	<0.01	0.02	0.09	0.06	0.10	0.05
Cu, ug/l	1.85	2.31	3.11	1.90	2.71	2.31	2.43	3.92
As, ug/l	3.86	3.68	4.26	4.36	<1.5	<1.5	<1.5	<1.5
Se, ug/l	6.30	10.02	7.32	8.12	13.77	14.85	11.35	6.74

[a]S = approximately half a meter below surface
B = approximately half a meter above bottom
[b]mean of triplicate samples
[c]Exceed USEPA criteria

Sediment

Sediment samples were collected twice for metals analysis. Number of replicates for the first sampling time was 8 for Stations 1 and 2 and 7 for Stations 3 and 4. For the second sampling time 2 replicates were used per station. Means were compared using the method of Least Squares Means at the 95 percent confidence level. All levels of metals in sediment are expressed as wet weights. For cadmium, Station 1 and 2 levels were significantly higher than Station 4, and Station 1 was also different from 3. For iron, levels in Station 1 and 2 sediment were significantly higher than levels in Stations 3 and 4. Lead levels in sediment from Station 1 were significantly higher

than levels from Stations 2, 3 and 4. For zinc, levels in Station 1 sediment were higher than levels at Stations 3 and 4. No significant differences in copper levels were found for sediment. Levels of iron, lead, zinc and cadmium in sediment from Station 1 were lower than previous levels reported by McCormick for a similar area [3]. None of the levels exceed the United States Geological Survey "Alert Levels" for sediments [38].

Table 22. Levels of metals in sediment from Grand Lake

Sample Station	N, total # replicates	Mean	Standard Error	Element	[a]USGS "Alert Levels"
1	10	1356.6	112.3	Cadmium	20,000
2	9	930.1	113.8	ug/kg	
3	10	577.2	112.3		
4	9	491.5	113.9		
1	10	11.3	0.54	Iron	------
2	9	11.0	0.54	g/kg	
3	10	7.8	0.54		
4	9	7.8	0.54		
1	10	16.1	0.97	Lead	500
2	9	11.9	0.99	mg/kg	
3	10	9.1	0.97		
4	9	10.5	0.99		
1	10	322.2	23.11	Zinc	5,000
2	9	257.9	23.44	mg/kg	
3	10	198.4	23.11		
4	9	208.5	23.44		
1	10	8472.2	795.0	Copper	2,000
2	9	7153.5	806.3	ug/kg	
3	10	5097.9	795.0		
4	9	6174.9	806.3		

[a] United States Geological Survey

Fish tissue

Levels of cadmium were measured for gizzard shad caught at three stations on the lake: 1, 2 and 4. Sample size was 6, 8 and 8, respectively. All levels of metals in tissue are expressed as wet weights. Mean levels of cadmium were determined and compared via Tukey's Method of Multiple Comparisons [36]. No significant difference in liver or kidney cadmium levels was found (Tables 23 and

24).

Average levels of lead in liver and kidney tissue were compared and no significant difference was found between fish caught from Station 1 and those from Station 4.

Average levels of zinc in livers from fish collected from Station 1 were significantly higher than levels in fish collected at Station 4, 93.39 and 22.76 mg/kg, respectively. Levels in Station 2 fish livers were also significantly higher with an average value of 51.24 ug/kg. For kidney tissue, levels of zinc in fish collected from Station 1 were significantly higher than levels of fish from Station 4, with values of 262.25 and 77.63 mg/kg, respectively (Table 24).

Table 23. Levels of metals in Gizzard Shad livers from Grand Lake

Sample Station	N # of Fish	Mean	SD	SEM	Element
1	6	0.54	0.45	0.18	Cadmium
2	8	0.23	0.12	0.04	mg/kg
4	8	0.52	0.38	0.13	
1	6	1.97	2.41	0.98	Lead
2	8	1.04	0.60	0.21	mg/kg
4	8	0.43	0.41	0.15	
1	6	[a]93.39	32.79	13.39	Zinc
2	8	[a]51.24	21.27	7.52	mg/kg
4	8	22.76	7.83	2.77	

[a] Significant at p = 0.05 level

Table 24. Levels of metals in Gizzard Shad kidneys from Grand Lake

Sample Station	N # of Fish	Mean	SD	SEM	Element
1	6	0.45	0.51	0.21	Cadmium
2	8	0.12	0.09	0.03	mg/kg
4	8	0.36	0.21	0.08	
1	6	7.44	9.27	3.78	Lead
2	8	2.54	1.85	0.65	mg/kg
4	8	0.79	1.00	0.35	
1	6	[a]262.25	177.28	72.37	Zinc
2	8	176.38	51.63	18.26	mg/kg
4	8	77.63	59.34	20.98	

[a]Significant at p = 0.05 level

Table 25. USEPA reference fish tissue samples

Element	[a]known conc. mg/kg	observed conc. mg/kg	95% Confidence Interval
Zinc	43.6	42.4	35.5 - 51.7
Cadmium	0.16	0.15	[b]MDL - 0.32
Copper	2.21	2.70	0.93 - 3.49
Lead	0.26	0.15	[b]MDL - 1.10

[a]mean of four replicates
[b]Maximum detectable limit

Levels of zinc are significantly higher in shad from the upper end of the lake compared to shad from the lower end. Whether or not these levels are high enough to hinder reproductive success, thus causing a change in the population structure, is difficult to determine. Migration of fish from the lower end of the lake would probably compensate for any temporary effect, making an assessment based upon density of standing crop measures of fish difficult.

CHAPTER VI

SUMMARY

Levels of metals in the sediments of the upper stations are higher than in the lower stations. This is demonstrated by both fish and water levels: higher levels of zinc in shad from Station 1 than Station 4 and higher levels of suspended and dissolved metals in Station 1 column water than Station 4.

However, the only toxicity observed in any of the organisms tested occurred with sediment extract from Station 4, indicating that the physical conditions of sediment from the upper stations are acting as a more effective trap for the metals. In general, levels of dissolved metals extracted at pH 10 are higher than those extracted at pH 4, independent of station location. This is probably due to sulfide chemistry. More metals will remain bound or in the non-ionic form at lower pH values, depending upon the amount of sulfides present.

APPENDIX

FIELD DATA

Table 26. Summary of field data for Station 1

Date	6-89		7-89		8-89		10-89	
Station, Depth Parameter	1S	1B	1S	1B	1S	1B	1S	1B
pH	7.9	7.7	7.9	7.6	6.4	7.9	8.0	--
Conductivity, uohms/cm3	390	390	262	261	256	253	320	--
Temperature, ^{o}C	27.5	23.8	24.0	24.1	27.0	27.0	16.9	14.2
Dissolved Oxygen, mg/l	9.1	1.2	4.9	0	6.7	6.6	11.9	0
Alkalinity	122	118	59	60	72	64	124	166
Hardness	168	170	112	105	112	100	160	166
Secchi Disk ınches	10		4		14			
Turbidity, N.T.U.	36	58	126	146	39	64	22	81

Table 27. Summary of field data for Station 2

Date	6-89		7-89		8-89		10-89	
Station, Depth Parameter	2S	2B	2S	2B	2S	2B	2S	2B
pH	8.5	6.6	--	--	9.0	6.9	8.0	--
Conductivity, uohms/cm3	270	270	--	--	301	--	320	360
Temperature, ^{o}C	26.0	16.0	24.5	19.4	28.3	17.8	20.4	14.2
Dissolved Oxygen, mg/l	8.0	0	6.9	0	10.0	0	9.9	0
Alkalinity	87	108	110	96	82	112	82	86
Hardness	117	136	146	144	118	142	104	146
Secchi Disk	14		23		25		18	
Turbidity, N.T.U.	18	45	13	46	76	290	22	81

Table 28. Summary of field data for Station 3

Date	6-89		7-89		8-89		10-89	
Station, Depth Parameter	3S	3B	3S	3B	3S	3B	3S	3B
pH	8.1	6.6	--	--	8.3	6.5	6.9	7.1
Conductivity, uohms/cm3	269	268	250	--	270	230	262	291
Temperature, ^{o}C	23.1	15.5	24.5	14.8	24.6	15.0	22.8	18.8
Dissolved Oxygen, mg/l	9.1	0	7.2	0	9.6	0	7.0	0.5
Alkalinity	86	98	134	110	88	102	74	82
Hardness	110	134	120	136	118	130	110	114
Secchi Disk	56		49		47		58	
Turbidity, N.T.U.	3.8	11.0	3.9	5.2	7.0	11.0	8.0	58

Table 29. Summary of field data for Station 4

Date	6-89		7-89		8-89		10-89	
Station, Depth Parameter	4S	4B	4S	4B	4S	4B	4S	4B
pH	8.4	7.8	--	--	8.3	7.1	8.2	7.5
Conductivity, uohms/cm3	250	260	--	--	256	268	266	258
Temperature, °C	25.5	14.0	--	--	23.9	11.9	22.8	17.5
Dissolved Oxygen, mg/l	12.0	0.5	--	--	8.2	0	8.2	0
Alkalinity	72	90	86	100	81	105	77	79
Hardness	108	124	104	136	123	130	112	113
Secchi Disk	51		72		55		71	
Turbidity, N.T.U.	4.1	9.4	4.2	3.3	5.2	6.7	5.0	36

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